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Prenatal Exposure to Pesticides: A Feasibility Study Among Migrant and Seasonal Farmworkers

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Background *Migrant and seasonal farmworkers have a high potential for pesticide exposures, yet are rarely included in epidemiologic studies. This study examined the feasibility of assessing prenatal exposures to pesticides and other compounds in pregnant Hispanic farmworkers.*

Methods *Nine women completed a survey about work experiences during pregnancy. Maternal urine, cord blood, and placenta samples were obtained at delivery for analysis of 51 analytes, including 6 phenoxy acid or triazine herbicides, 21 organochlorine insecticides, 10 PCBs, and 14 volatile organic compounds.*

Results *Seven of 51 analytes were found in the biological samples. DDE, DDT, dichlorbenzene, toluene, trimethylbenzene, and endosulfan sulfate were detected in cord blood samples, and 2,4-D in urine from one or more women.*

Conclusions *We documented the feasibility of following farmworkers to assess in utero exposure to pesticides and other contaminants, and demonstrated exposure to these compounds. Difficulties in measuring pesticides with short half lives were noted.* Am. J. Ind. Med. 40:578–585, 2001. © *2001 Wiley-Liss, Inc.*

KEY WORDS: prenatal; farmwork; migrant; pesticides; cancer; occupation; Hispanic; Texas

INTRODUCTION

Pesticides are a broad class of chemicals used for pest control and are classified by formulation, target pests, mode of action, or chemical structure [Stevenson, 1995]. While there are many benefits to pesticide application, pesticide exposures have been associated with a wide variety of adverse health outcomes. Acute health effects include irritant effects, systemic poisoning, and contact dermatitis. Chronic health effects include cancer, neurotoxicity, birth defects, and adverse reproductive effects [Sharp and Eskenazi, 1986; Moses, 1993]. A number of studies have linked pesticides to childhood cancer [Zahm and Ward, 1998]. Some epidemiologic studies have reported an association between childhood cancer risk and parental exposure to pesticides during pregnancy [Zahm and Ward, 1998]. The transplacental carcinogenesis of diethylstilbestrol [Giusti et al., 1995] illustrates the vulnerability of the fetus to the effects of in utero exposure. Prenatal exposure appears particularly hazardous [Rogan et al., 1986; Jacobson et al., 1990; Yu et al., 1991], although, one small study of prenatal DDE exposure showed no relation to preterm delivery [Berkowitz et al., 1996]. Animal studies demonstrating the transplacental transfer of genotoxins with subsequent teratogenic and carcinogenic effects also raise concern about in utero exposure to pesticides [Autrup, 1993]. The International Agency for Research on Cancer concluded that there was limited or sufficient evidence for

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carcinogenicity of about half of the pesticides evaluated in experimental studies [IARC, 1987].

There is a paucity of research on occupational and environmental hazards affecting minority women, particularly those of Hispanic ethnicity [Dula et al., 1993]. People of color and low-income groups experience a disproportionate burden of pesticide exposure and related health effects, and yet are rarely studied [Moses et al., 1993]. Women who work as migrant or seasonal farmworkers have a particularly high potential for exposure to pesticides.

The objective of this study was to examine the feasibility of using biological monitoring to assess prenatal exposures to pesticides and other compounds in a Hispanic population of migrant and seasonal farmworkers.

MATERIALS AND METHODS

Study Population

Pregnant farmworkers who received prenatal care and delivered at Holy Family Services Birth Center (HFSBC) in Weslaco, Texas between November 7, 1997 and March 30, 1998 were eligible to participate in the study. Fourteen farmworkers received prenatal care at HFSBC during this period, but five were ineligible because they delivered after March 30, were transferred to a hospital during delivery, or delivered at another facility due to other reasons (such as a change in residence). The remaining nine farmworkers agreed to participate in the study.

Weslaco is located in Hidalgo County, one of the counties in the Rio Grande Valley of Texas that lies on the Texas-Mexico border. Hidalgo County has the largest estimated number of farmworkers and dependents of any county in the state [USDHHS, 1990]. Significant opportunities exist for environmental exposure to pesticides in this region both from domestic use and crossover from the Mexican side of the border. The timing of the study (late fall, 1997 through winter, 1998) coincided with the farmworkers' recent seasonal return from migrating for farmwork. HFSBC serves women along the Texas-Mexico border without regard to socioeconomic status. This birth center is accredited through the Commission for the Accreditation of Freestanding Birth Centers and licensed through the Texas Department of Health. The staff nurse-midwives are certified by the American College of Nurse-Midwives and licensed as advanced practice nurses by the Board of Nurse Examiners for the State of Texas.

Questionnaire Administration and Biological Sample Collection

Informed consent to participate in this study was obtained from the nine study subjects. During a prenatal visit close to the projected time of delivery, a brief questionnaire was administered in Spanish or English by one of the three trained staff members at HFSBC. The questionnaire solicited information about demographics, pregnancy history, and work activities in or near fields during the current pregnancy. Each woman agreed that three types of biological samples (cord blood, maternal urine, and placental sections) would be obtained at the time of delivery upon verbal re-consent.

Due to the pilot nature of this feasibility study, limited time and funding precluded a prospective study design that would permit data collection over the course of pregnancy. Therefore, the decision was made to evaluate primarily compounds with long half-lives that could be expected to be measurable weeks or months after exposure occurred (i.e., at delivery). However, this approach did enable us to ascertain protocols that would be acceptable to this population, with regard to both questionnaire completion and the provision of biological samples for exposure assessment.

The biological specimens were collected according to protocols that were explained during a training session with the nurse-midwives responsible for collecting the samples at delivery. The protocols were also documented and posted in each delivery area at HFSBC. The maternal urine samples were collected just prior to delivery in a sterile specimen cup. The samples were immediately poured into a coded laboratory jar until the jar was approximately three-fourths full. The jar was labeled according to the study protocol and frozen until ready for shipment to the laboratory.

Once the umbilical cord was cut after delivery, blood from the cord was collected in a 30-cc syringe. The blood in the syringe was delivered into serum-separating Vacutainers® and Vacutainers® containing EDTA. The EDTA Vacutainers® were labeled according to the study protocol, without centrifugation. Specimens were refrigerated until shipment to the laboratory.

Three tissue specimens were extracted from the non-cord side of the placenta according to a protocol from the Centers for Disease Control and Prevention (CDC) Division of Environmental Health Laboratory Sciences (CDC, personal communication). Using the analogy of a "clock," the nurse-midwives cut pieces weighing approximately 5 grams from the 12, 2, and 5 o'clock positions. The 12 o'clock section was taken from the edge and included both maternal and fetal sides. The 2 o'clock piece was cut very close to the center, and the 5 o'clock sample was removed about midway between the edge and the center of the placenta. Care was taken to avoid the cotyledons, and no preservatives were used. The three specimens were placed in separate glass jars and labeled according to the study protocol. Placental specimens were stored in a refrigerator until ready for shipment.

All specimens were stored in appropriate containers that were pre-labeled except for the date of collection, study identification number, and placenta section site, which were

documented at the time of sample collection by the nurse-midwives responsible for this task. After all three types of specimens were collected and stored for a single study participant, they were sent immediately with an ice pack via overnight express mail to Accu-Chem Laboratories (a USDHHS certified laboratory in Richardson, TX).

Laboratory Analysis

Table I shows the compounds that were measured in each type of sample and the limits of detection for each analyte. Two analysis panels (phenoxy acid and triazine herbicides) were run on the maternal urine samples. Three analysis panels were run on the cord blood specimens: chlorinated pesticides and PCBs in serum, and volatile organic compounds (VOCs) in whole blood. These three panels were also run on the 12 o'clock placental samples. The PCB panel was also run on the 2 o'clock placental sample to examine the consistency of the findings between the two placenta sample locations. The 5 o'clock sample was collected with the intention to analyze as a replacement, if needed, although this situation did not occur.

Phenoxy acid herbicides and triazine herbicides were analyzed in small aliquots of maternal urine following derivatization to their methyl ester forms. High resolution gas chromatography and mass spectrometry (GC/MS) were used to identify and quantify urinary levels of these herbicides. The method of Dale et al. [1966] was employed for the analysis of organochlorine pesticides and PCBs in cord serum. High resolution gas chromatography coupled with detection by electron capture detector (GC/ECD) was used to quantify and identify each compound. For VOCs in the cord blood, the refrigerated blood samples were allowed to reach room temperature prior to analysis. The volatiles were separated using thermal desorption onto a silica capillary column before entering the mass spectrometer for analysis where the samples were qualified and quantified. To analyze the placental samples for chlorinated pesticides, PCBs and VOCs, the tissues were minced and chemicals extracted prior to analysis by GC/MS.

Data Analysis

After the questionnaires and laboratory results were received at the University of Texas School of Public Health,

TABLE I. Compounds Measured and Analytic Limits of Detection

Specimen type	Compounds measured	Detection limit (ppb)
Maternal urine	Phenoxy acid herbicides (n = 3)	20.0
	2,4-D	
	2,4,5-TP	
	2,4,5-T	
	Triazine herbicides (n = 3)	
	Atrazine	20.0
	Simazine	20.0
	Propazine	100.0
Cord blood serum/Placenta	Chlorinated pesticides (n = 21)	0.3
	Aldrin	
	BHC (alpha, beta, gamma, delta)	
	Chlordane (alpha, gamma)	
	DDD	
	DDE	
	DDT	
	Dieldrin	
	Endosulfan II	
	Endosulfan sulfate	
	Endrin	
	HCB	
	Heptachlor	
	Heptachlor epoxide	
	Methoxychlor	
	Mirex	
	Oxychlordane	
	Trans-nonachlor	
	PCBs (n = 10 cogeners)	0.3
	23′44 tetra	
	23′44′5 penta	
	22′44′55′ hexa	
	233′44 penta	
	22′344′5 hexa	
	22′344′5′ hexa	
	22′344′5′6 hepta	
	22′33′44′6 hepta	
	22′344′55′ hepta	
	22′33′44′5 hepta	
Cord blood (whole)/Placenta	Volatile organic compounds (n = 14)	0.5
	Benzene	
	Bromodichloromethane	
	Carbon tetrachloride	
	Chloroform	
	Dichlorobenzenes	
	Dichloromethane	
	Ethylbenzene	
	Styrene	
	Tetrachloroethylene	
	Toluene	
	1,1,1-Trichloroethane	
	Trichloroethylene	
	Trimethylbenezenes	
	Xylenes	

the data were input into FoxPro® database files for management and analysis. We calculated proportions for variables related to demographics and work history. To examine the association between work history variables and laboratory findings, we examined the data graphically. Statistical analysis was not performed due to the small sample size.

Where possible, concentrations of pesticide metabolites in maternal urine and of volatile organic compounds (VOCs) in cord whole blood were compared to published levels observed in urine and adult whole blood, respectively, in a subset of the larger National Health and Nutrition Examination Survey III (NHANES III), conducted between 1988 and 1994 [Ashley et al., 1994; Hill et al., 1995]. The adults ranged in age from 20 to 59 and were diverse in terms of sex, race, urban/rural residence, and geographic region. Although they were not a random sample of the U.S. population [Needham et al., 1995], they are the best available U.S. general population for comparison.

RESULTS

Questionnaire Data

All the nine eligible farmworkers agreed to participate, and all of the subjects answered the questionnaire and provided biological samples. The average age of the subjects was 28 years (range = 18–40 years). All resided permanently in the Valley near farming fields, and six (67%) had migrated for farmwork prior to their pregnancy for a period ranging from 2 to 25 years (median = 8.5 years). The other three women performed farmwork locally in the Valley.

Seven women (78%) worked in the fields for pay during their current pregnancy, and two (22%) helped spouses and/or family members in the fields for no pay. Of the seven women who worked for pay, five performed both migrant and non-migrant farmwork (i.e., performed farmwork while remaining at their permanent residence), one did only migrant farmwork, and one did strictly non-migrant farmwork. Over half of the women (56%) provided care for children as they played in or near the fields, and four (44%) performed farming tasks near fields, such as handling and packaging crops. All the nine women worked or participated in activities in or near farm fields during their pregnancies for periods ranging from 2 to 8 months (Table II). One woman worked only during the first trimester of pregnancy, one worked only during the second trimester, one worked during the first two trimesters, and the other six worked during all three trimesters. Four women (44%) reported that they had in some way changed their work or activities near the fields during their pregnancy; the most frequent change was to stop working. Overall, these nine women worked for an average of 5.8 months during their pregnancy.

TABLE II. Number of Months Farmworker Women Worked During Pregnancy

Number of months worked during pregnancy	Number of women (Percent)
1	0 (0)
2	1 (11)
3	1 (11)
4	1 (11)
5	1 (11)
6	1 (11)
7	0 (0)
8	4 (44)
9	0 (0)
Total	9 (100)

The study population reported working with or conducting activities near a large number of crops. With the exception of one study participant, who reported watching her children as they played in or near only one crop, all women worked with or near at least four different crops during their pregnancy. Two women worked with five crops and two worked with six. Overall, these nine women reported working with 28 different crops during their current pregnancy.

Analyses of Biological Samples

All women had detectable levels of at least one compound in their biological samples. The number of analytes detected per woman ranged from one to five (Table III). The only compound detected in the maternal urine samples was 2,4-D (Table IV). The measured level of 2,4-D of 120 ppb in one farmworker was more than three times higher than the maximum level observed in urine from the NHANES III participants (37 ppb) [Hill et al., 1995].

Three organochlorine pesticides (DDE, DDT, and endosulfan sulfate) and three VOCs (dichlorobenzenes, toluene, and trimethylbenzene) were detected in the

TABLE III. Number of Compounds Detected in Biological Samples

Number of detected compounds	Number of women	Percent
1	1	11
2	5	56
3	0	0
4	2	22
5	1	11
TOTAL	9	100

TABLE IV. Analytes Detected in Maternal Urine and Umbilical Cord Blood*

Type of sample	Analyte	No. of subjects detected	Range of detected values (ppb)
Maternal urine	2,4-D	1	120
Cord (serum)	DDT	1	0.7
	DDE	9	0.5–3.3
	Endosulfan sulfate	1	0.9
Cord (whole blood)	Dichlorobenzenes	3	0.7–2.1
	Toluene	1	2.1
	Trimethylbenzene	7	1.2–3.9

*No analytes were detected in the placenta samples.

umbilical cord blood samples. DDE was detected in cord blood serum samples from all the nine women at levels ranging from 0.5 to 3.3 ppb, with a median of 1 ppb, and endosulfan sulfate was detected in one of the farmworkers at a level of 0.9 ppb. Toluene was detected in one umbilical cord whole blood sample at a concentration of 2.1 ppb, which exceeds the 95th percentile concentration of 1.5 ppb in regular adult blood from NHANES III [Ashley et al., 1994]. Although the frequency of detection of toluene in NHANES III (over 95%) was much higher than in our study, the detection limit for toluene in NHANES III (0.092 ppb) was lower than in our study (0.5 ppb)[Ashley et al., 1994]. Dichlorobenzenes were found in three samples at levels ranging from 0.7 to 2.1 ppb. Only 1,4-dichlorbenzene was measured in NHANES III, and it was found at higher levels than were the dichlorobenzenes in our study; the 95th percentile in NHANES III was 9.2 ppb and the maximum value was 12.0 ppb [Ashley et al., 1994]. None of the analytes was detected in the placenta samples. A graphical examination of the data revealed no clear association between the number of positive laboratory findings and age or the number of months worked during pregnancy (data not shown).

DISCUSSION

The weakest link in many occupational and environmental epidemiologic studies is exposure assessment. One approach often used to estimate exposures in epidemiologic studies is imputation based on questionnaire data such as job title or task. Job exposure matrices are usually applied to non-agricultural jobs but have been reported to be useful for epidemiologic studies of agricultural chemicals as well [London and Myers, 1998; Ward et al., 2001, this issue]. While job exposure matrices can enhance questionnaire data, they are limited by exposure variability within the same job/task category [Kauppinen et al., 1998]. Biological measurements are another approach for assessing exposures. Biologic measures of exposure may not always be the gold standard or even the best indicator of relevant exposure, depending on the chemical's biologic half-life and the latency of the disease of interest [Schulte, 1993, 1995]. Studies of health effects due to pesticide exposures in utero have the advantage of a relatively short time period of interest for exposures, but even nine months is long compared to the biologic half-lives of many pesticides, which are often measured in hours or days.

In this study, we assessed potential exposures via questionnaires and through analyses of contaminants in maternal urine, cord blood, and placental tissue samples. This study clearly demonstrates the feasibility of obtaining questionnaire data during pregnancy and biological samples at delivery from migrant and seasonal farmworkers. While the number of women participating in our study was small, the study documented maternal and fetal exposure to pesticides and other compounds in the migrant farmworker population. One subject, for example, had detectable levels of endosulfan sulfate in blood and a measured concentration of 2,4-D in urine. Endosulfan sulfate is a metabolite of the organochlorine insecticides endosulfan I and II, which are used on cotton, fruits, and vegetables [Stevenson, 1995]. 2,4-D is a phenoxyacid herbicide used on a variety of crops as well as on golf courses and residential lawns [Stevenson, 1995]. Since 2,4-D has an estimated half-life of about 10–20 hr in living organisms [Oregon State University, 1996a] and most endosulfan is eliminated from the body within a few days to a few weeks of exposure [Naqvi and Vaishnavi, 1993], detectable levels of these contaminants in biological fluids at delivery indicates that this woman was exposed late in her pregnancy. The extent to which such exposures may cause potentially adverse reproductive effects in humans is not known. However, studies have found that endosulfan can cause developmental and teratogenic effects in animals [Naqvi and Vaishnavi, 1993]. Reproductive and teratogenic effects of 2,4-D exposure have also been observed in animals, but only at high doses [Oregon State University, 1996a].

While pesticide exposure to the fetus may be inferred by sampling maternal blood and urine during pregnancy, the sampling of cord blood is a more direct way of assessing fetal exposure. Cord blood samples have been used to assess fetal exposure to organochlorine pesticides and PCBs [Eckenhausen et al., 1981; Saxena et al., 1983; Skaare et al., 1988; Kanja et al., 1992; Hagmar et al., 1998], as well as heavy metals [Ong et al., 1993; Yang et al., 1997]. All the nine umbilical cord serum samples had detectable levels of DDE, a metabolite of DDT. The DDE levels observed in this study (0.5–3.3 ppb) were similar to levels typically observed by the CDC laboratory of about 2 ppb in regular adult serum samples from the general population (Needham,

personal communication). Although DDT was banned in the U.S. in 1972 [ATSDR, 1989] and levels have decreased substantially since then [Needham et al., 1996], we had hypothesized that DDE would be found at higher levels than those observed in the study subjects because DDT continues to be used in Mexico to combat malaria in sanitation campaigns [Lopez-Carrillo et al., 1996], and ongoing exposure could result from living in close proximity to the Mexican border or from working in Mexico. However, continued application of DDT in the study site along the Texas-Mexico border was not documented.

Eight subjects had detectable levels of VOCs in the umbilical cord samples (toluene, dichlorobenzenes, and/or trimethylbenzenes). Toluene, a solvent, is present in gasoline, paints, and adhesives [EPA, 1994a], and is an ingredient in some pesticides [Briggs, 1992]. Dichlorobenzenes are used as solvents, insecticides, and in deodorizers [NIEHS, 1985; ATSDR, 1993]. Trimethylbenzene is used as an additive to gasoline, paints, and cleaners, and as an additive to pesticides [EPA, 1994b]. The U.S. Environmental Protection Agency (EPA) lists trimethylbenzene as an inert ingredient in 18 pesticides (Dr. Jerome Blondell, EPA, July, 1998), but it may also be a contaminant from the plastic vacutainers used in this study for the collection of cord blood [EPA, 1994b; Cardinali et al., 1995]. None of the compounds was detected in placental tissue, despite the presence of several compounds in cord blood. The correlation between cord blood and placental levels appears to be dependent on the chemical nature of the compounds being evaluated [Saxena et al., 1981; Ando et al., 1985]. For example, Ando et al. [1985] demonstrated a significant correlation between hexachlorobenzene (HCB) concentrations in placenta and maternal blood and milk. On the other hand, Ando et al. [1986] were not able to find a significant correlation between the concentrations of various PCBs found in placental tissue and in cord blood. They suggested that these differential effects were due to placental selectivity toward the transfer of HCB and PCBs across the placental barrier. This selectivity may be due to differences in the lipophilic nature of the compounds since the more highly chlorinated PCB congeners correlated more significantly than did the lower chlorinated compounds [Ando et al., 1986]. However, in addition to the type of compound, there may be other contributing factors that complicate the interpretation of placental pesticide concentrations in relation to that found in cord blood such as sampling of placental tissue.

Despite the fact that all of the study subjects did farm work during their pregnancy and most worked with a variety of crops, most of the analytes were not detected in the biological samples. There are several possible explanations for this. First, hundreds of pesticides are used in agriculture and we have no way of knowing which, if any, were used on the fields in which these women worked. Only five of our analytes (three herbicides: 2,4-D, atrazine, and simazine; and two insecticides: endosulfan and methoxychlor) were used in 1997 in the major producing states on the crops with which the women reported working, and the percent of acreage treated with these pesticides was typically small [USDA, 1998a, 1998b, 1999]. The women may have been exposed to other, more widely-used pesticides that we did not test for due to budget constraints and the likelihood of non-detection due to short half-lives. Similarly, it is also possible that exposures occurred during earlier periods of pregnancy and that levels of contaminants were not detectable at the time of delivery due to the short half-lives of many of the contaminants that were examined [Naqvi and Vaishnavi, 1993; Oregon State University, 1996b, 1996c; Cummings, 1997]. While the use of personal protective equipment would have reduced exposure, personal protective equipment is not used extensively in this population and probably is not an important factor [London and Myers, 1988].

Although this study demonstrated the feasibility of obtaining questionnaire data during pregnancy and biological samples at delivery from migrant and seasonal farmworkers, it also illustrated the difficulties associated with using biological monitoring to assess maternal and fetal exposures to pesticides and other compounds. First, the suitability of biologic measures of exposure is heavily dependent on the underlying kinetics of the contaminant in the body, and is likely to differ greatly among compounds [Droz et al., 1991; Rappaport et al., 1995]. As noted earlier, compounds that are relatively short-lived in the body may offer limited utility unless specimens are collected during or shortly after exposure occurs. This would necessitate sampling of maternal blood and/or urine throughout pregnancy, which would be difficult under most circumstances and infeasible during periods of migration. Secondly, biological monitoring may not be suitable when the nature of the work makes it difficult to pinpoint a priori which exposures among a broad range of possible contaminants are more likely than others.

In conclusion, despite the emphasis on and utility of molecular epidemiologic techniques to improve exposure assessment [McMichael, 1994], the continued importance of descriptive questionnaire-based methods to develop qualitative or semi-quantitative measures of exposure should not be discounted. Future studies will need to rely on questionnaire data, which are less expensive to collect and provide information on possible sources and duration of exposure, timing in relation to pregnancy, job tasks, and collection of data on potential confounders. However, exposures during the prenatal period should continue to be assessed by whatever methods are deemed the most suitable so that critical exposures during this earliest time period can be carefully considered in health effects studies of children in agricultural communities.

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